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COOPERATIVE AGREEMENT NUMBER DAMD17-95-2-5005

TITLE: Development of Recombinant Expression Systems for the Production of Malaria Antigens

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REPORT DATE: March 1998

TYPE OF REPORT: Final

PREPARED FOR: Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	March 1998	Final (1 Mar 95 - 28 Feb 98)

4. TITLE AND SUBTITLE
Development of Recombinant Expression Systems for the Production of Malaria Antigens

5. FUNDING NUMBERS
DAMD17-95-2-5005

6. AUTHOR(S)
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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200*

Malarial erythrocytic-stage antigens produce humoral immunity by inducing protective antibodies to epitopes on surface antigens on the parasites. The merozoite surface protein-1 (MSP1) of *P. falciparum* is a major malaria erythrocytic-stage vaccine candidate. MSP1 may play a role in binding and/or infection of erythrocytes by merozoites (parasites) during red blood cell invasion. The C-terminal fragment of MSP1, $MSP1_{42}$, is highly conserved among all known strains of *P. falciparum*. However, it is folded into a complex structure containing six possible disulfide bridges. Immunization with recombinant proteins that have native-like conformations may elicit long-lasting protective immunity that mimics the natural development of humoral immunity. We have used a novel bacterial expression system that utilizes tightly regulatable transcription and translation signals to control expression of potentially toxic heterologous proteins. Several plasmids encoding $MSP1_{42}$ were constructed and tested for their ability to express recombinant protein. These constructs were designed for purification using Ni^{+2}-chelating chromatography. Our results show that the recombinant proteins contained conformationally similar structures to the native parasites. The objectives were to develop recombinant $MSP1_{42}$ molecules that were structurally correct, (b) to develop fermentation and purification processes that could be scaled-up for large-scale processes, and ultimately (c) to advance these products into human clinical trials.

14. SUBJECT TERMS
Malaria, Bacterial Expression, Merozoite Surface Protein, Conformation, , Affinity Chromatography

15. NUMBER OF PAGES
26

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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NA In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

NA In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

NA In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

Robert Cayer 3/24/98

PI - Signature Date

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INTRODUCTION

The mosquito-born parasite *Plasmodium falciparum* is the leading cause of clinical malaria in man. Malaria can result in serious clinical illness and if untreated can lead to death. Approximately 300 million cases of malaria are reported yearly, with 3 million deaths. Efforts toward the development of effective controls against the mosquito vector and the parasite have lead to wide spread pesticide and drug resistance. As the anti-vector and anti-parasite approaches failed, efforts have focused on malaria vaccine development as an alternative approach.

Vaccine candidates have been identified from each of the parasite's developmental stages. A leading erythrocytic stage vaccine candidate is the major merozoite surface protein-1, MSP1, [Diggs, *et al.*, 1993]. Vaccines that are derived from malaria erythrocytic-stage antigens like MSP1 are of special interest because erythrocytic stages are the only confirmed targets of natural immunity among individuals from malaria endemic regions. The development of an efficacious erythrocytic stage malaria vaccine from MSP1 or the C-terminal fragment ($MSP1_{42}$) has the potential to protect non-immune individuals. Since malaria-naïve individuals do not possess partial immunity developed through life-long exposures, immunization with an $MSP1_{42}$ vaccine could induce the development of antibodies that are qualitatively comparable to those developed from natural malaria exposures. The mechanism of protection induced by an erythrocytic stage malaria vaccine would be mediated through the development of specific protective antibodies to proteins on the surface of parasites. Antibodies raised to parasite surface proteins would lead to an inability of the erythrocytic stage parasites

(merozoites) to re-invade new erythrocytes. The putative mode of action of these antibodies is to bind to the surface of the merozoites and block their ability to associate with, and invade erythrocytes, or to interfere with biochemical events associated with invasion. The effect of blocking invasion would be to reduce the potential amplification of parasites in the bloodstream and thus reduce the overall parasitic load and severity of disease. Therefore the development of specific antibodies to erythrocytic stage antigens like MSP1 could reduce the likelihood of serious illness and disease in malaria-naïve individuals.

Although it has been extensively investigated, [Holder, *et al.*, 1988, Miller, *et al.*, 1993], MSP1's function is not well understood [Holder and Blackman, 1994]. MSP1 is initially synthesized as a large 195 kDa precursor protein. Proteolytic processing of this protein yields products with nominal molecular masses of 83, 28-30, 38-45 and 42 kDa. Merozoite-specific antibodies that recognize these processed forms have been identified [Holder and Freeman, 1984; Lyon, *et al.*, 1986; Holder, *et al.*, 1987]. A non-covalent complex of proteolytic fragments forms on the surface of merozoites [McBride and Heidrich, 1987; Lyon, *et al.*, 1987] and remains attached to the merozoite surface through the C-terminal 42 kDa fragment ($MSP1_{42}$). At the time of erythrocyte invasion, $MSP1_{42}$ is processed further to a 33 kDa fragment and a 19 kDa C-terminal fragment ($MSP1_{19}$) [Blackman, *et al.*, 1991]. This event results in the shedding of the non-covalently associated protein complex from the merozoite surface leaving only the 19 kDa fragment surface-anchored through N-glycosylphosphatidylinositol [Haldar, *et al.*, 1985]. During the invasion process, only $MSP1_{19}$ is present on ring forms in the newly invaded erythrocyte [Blackman, *et al.*, 1990]. The apparent structure of $MSP1_{19}$ is

rather complex, containing 12 cysteines within a span of 100 amino acid residues, and may be arranged as two tandem domains homologous with epidermal growth factor (EGF) [Blackman, *et al.*, 1991]. Each putative EGF-domain contains six cysteine residues that would form three disulfide bridges per domain, though neither the number nor the pattern of the disulfides has been determined.

Development of specific antibody responses to native MSP1 molecules requires that important conformational epitopes be present on the surface of these molecules. Several lines of evidence support the use of MSP1, and especially the C-terminal fragments, $MSP1_{42}$ and $MSP1_{19}$, as one component of an erythrocytic stage malaria vaccine. First, $MSP1_{19}$-specific monoclonal antibodies inhibit *P. falciparum* growth *in vitro,* [Blackman, *et al.*, 1990], or passively protect mice against infection with *P. yoelii,* [Majarian, *et al.*, 1984; Ling, *et al.*, 1994]. Second, immunization of monkeys with native MSP1, [Siddiqui, *et al.*, 1987], baculovirus-expressed recombinant $MSP1_{42}$ [Chang *et al*, 1996], or *S. cerevisiae*-secreted recombinant $MSP1_{19}$ (EVE-$MSP1_{19}$) from *P. falciparum* [Kumar, *et al.*, 1995], can protect against a homologous challenge. Similarly, *E. coli*-expressed recombinant $MSP1_{19}$ from *P. yoelii,* [Holder, *et al.*, 1994; Burns, *et al.*, 1989] protects against a homologous murine challenge. And third, anti-sera raised against recombinant $MSP1_{42}$ [Chang, *et al.*, 1992], or $MSP1_{19}$ [Lyon and Haynes, unpublished] inhibit *P. falciparum* growth *in vitro*. The $MSP1_{19}$-specific monoclonal antibodies that either protect against infection *in vivo* [Burns, *et al.*, 1989], or inhibit parasite growth *in vitro* [Blackman, *et al.*, 1990], are specific for discontinuous epitopes since they do not react with disulfide-reduced $MSP1_{19}$ [McBride and Heidrich, 1987; Farley and Long,

1995]. Thus, a recombinant vaccine produced from this part of MSP1 may require correct disulfide-dependent conformation to elicit a protective antibody response.

Successful expression of heterologous proteins in *E. coli* can provide significant advantages over eukaryotic expression systems. Historically, expression in *E. coli* can lead to high levels of recombinant proteins. Bacterial expression has the advantage of being relatively inexpensive, and can provide ease of scaleable production and handling. However, some inherent disadvantages are that proteins expressed in bacteria are not post-translationally modified and heterologous proteins that require these modifications may have altered activities. Eukaryotic expression systems, such as yeast, baculovirus, or mammalian cells, can provide post-translational modifications, however, the modification may not be appropriate and yields can be poor. Therefore, heterologous expression of recombinant molecules must replicate the conformation and structure of these proteins to induce an appropriate immune response. Heterologous expression of some recombinant MSP1 molecules ($MSP1_{42}$ and $MSP1_{19}$) from eukaryotic expression systems, *i.e.* baculovirus and yeast, have lead to recombinant molecules that are either properly folded and expressed poorly or are mis-folded and expressed well, respectively. To circumvent these problems, novel bacterial expression systems were used to express the C-terminal $MSP1_{42}$ fragment. Although several C-terminal $MSP1_{42}$ or $MSP1_{19}$ constructs have been expressed from baculovirus, yeast and bacterial expression systems, difficulties exist with these constructs that limit their feasibility for development as vaccines (*i.e.* low expression levels, improper subunit folding and undesirable heterologous fusions at the N-terminus of the constructs, respectively).

The objective of this work was to develop erythrocytic stage malaria vaccine candidates that could elicit protective immune responses in volunteers in Phase I trials. Since the MSP1 molecule is a rather large protein, it is not practical to develop recombinant vaccines to the entire gene product. Furthermore, *in vitro* studies have shown that protective epitopes on MSP1 are contained within the C-terminal MSP1 cleavage product, $MSP1_{42}$. However, the C-terminal $MSP1_{19}$ contained within the $MSP1_{42}$ molecule, is highly cysteine-rich and is predicted to fold into a rather complex tertiary structure. Correct presentation of relevant epitopes on the C-terminal fragment is dependent on the proper folding and conformation of the $MSP1_{42}$ molecule. Our data show that these goals have been attained through the construction of recombinant DNA plasmids that encode the C-terminal $MSP1_{42}$ DNA sequence and their expression in specific *E. coli* hosts. Recombinant plasmids were transformed into *E. coli* BL21 (DE3) or AD494 (DE3), and recombinant proteins were expressed. To facilitate the purification and process development of the recombinant $MSP1_{42}$ molecules, a six-histidine (His_6) amino acid sequence tag was cloned at the amino terminus of the $MSP1_{42}$. The His_6 tag provided the capability for affinity purification of the $MSP1_{42}$ using nickel-chelating chromatography. Additional chromatography may include DEAE anion exchange chromatography to remove endotoxin and as a final "polishing" step.

Early process development of the $MSP1_{42}$ protein required fermentation at the ten-liter scale. Optimization of culture conditions and bacterial host expression of recombinant $MSP1_{42}$ will require controlled studies to measure the effects of variables induced by increasing the scale of the fermentation from one to two liters, to ten liters, and ultimately to thirty liters. The final development process, thirty liters, will provide

sufficient amounts of recombinant $MSP1_{42}$ protein for development of vaccines for testing in future human Phase I trials.

The production of recombinant molecules using DNA technology and large-scale fermentation processes has enabled the development of proteins in quantities otherwise impossible. Although previously it was difficult to use bacteria to express some complex eukaryotic proteins, recent advances in the design of bacterial strains and the development of tightly regulatable expression vectors have lead to their use in expression of some complex eukaryotic proteins.

The *E. coli* expression vector used for the construction of recombinant C-terminal fragment from MSP1 ($MSP1_{42}$), the pET32a vector from NOVAGEN, provides several advantages over other *E. coli* expression systems (Figure 1). Some features contained on the plasmid are; the *E. coli* thioredoxin gene, *trxA*, which confers increased thermal stability and cytoplasmic localization of fusion proteins; the antibiotic resistance gene, *bla*, for ampicillin selection; His_6-Tag sequences for metal-chelating affinity chromatography; the $lacI^q$ gene for constitutive expression of *lac* repressor; and the bacteriophage T7 promoter sequence. Target DNA's were cloned into the vector and transformed into T7 RNA polymerase-deficient hosts (*i.e.* BL21 or AD494), that allowed productive cloning in the absence of basal levels of transcription and translation. A chromosomally integrated T7 RNA polymerase under a *lac*UV5 promoter, regulates transcription of target sequences from the bacteriophage T7 promoter [chromosomally encoded in *E. coli* hosts AD494 (DE3) and BL21 (DE3)]. Induction of expression or de-repression at the *lac*UV5 and T7 *lac* promoter/operator site is mediated by the addition of the *lac* inducer, IPTG.

A major limitation for expressing some heterologous proteins in *E. coli* has been the requirement for proper disulfide bond formation. Normally, relatively high reducing potentials are maintained in the *E. coli* cytoplasm thus preventing disulfide bond formation in this compartment. In *E. coli*, protein disulfide bonds are normally formed following export into the periplasmic space. Since the $MSP1_{42}$ molecule contains six putative disulfide bonds, the development of a protective immune response from this region of MSP1 may require correct disulfide bond formation and folding. A commercially available *E. coli* host from NOVAGEN, AD494 (DE3) is deficient in cytoplasmic reduction potential and allows accumulation of oxidized forms of sulfhydryl groups on cytoplasmic proteins thus possibly circumventing this problem and allowing proper disulfide bond formation within the *E. coli* cytoplasm (Derman, *et al.*, 1993). However, some target proteins are more stable in the BL21 (DE3) expression host since they lack the *lon* protease and the *ompT* outer membrane protease which are involved in protein degradation during expression.

DEVELOPMENT OF RECOMBINANT $MSP1_{42}$ CONSTRUCTS

The construction of an appropriate $MSP1_{42}$ expression vector for use in expression and purification of a recombinant $MSP1_{42}$ molecule proceeded through a series of constructions that ultimately led to the final product, designated pET42AT(NK2), His_6-$MSP1_{42}$. The final construct, so developed, has been designed to meet the specifications required by the FDA for expression of a recombinant product from *E. coli*. The final His_6-$MSP1_{42}$ product contains a short N-terminal fusion on

$MSP1_{42}$ that encodes six histidine residues and 11 linker amino acids. The plasmid also contains a gene for tetracycline resistance selection.

The construction of a DNA vector expressing a *P. falciparum* 3D7 $MSP1_{42}$ molecule proceeded through the following steps. A full-length fusion with *E. coli* thioredoxin at the N-terminus of $MSP1_{42}$ was prepared by directional cloning using *BamHI* and *SalI* restriction sites in the multiple cloning region of the pET32a expression vector from Novagen (Construct #1). Positive clones were transformed into the highly regulatable T7 RNA polymerase expressing host, AD494 (DE3). Mini-induction experiments were conducted to optimize expression levels of several clones. In these experiments some variables that were investigated included induction temperature, concentration of inducer (IPTG), length of time of induction, and the influence of *E. coli* host background on levels of expression [BL21 (DE3) versus AD494 (DE3)]. These variables have been shown to affect the levels of expression and the partitioning of protein in either soluble or insoluble fractions. SDS-PAGE and immunoblotting analysis of un-induced and induced crude cell extracts showed that at 37°C, the full length fusion, *trxA-MSP1*$_{42}$ (Construct #1, pTRX42) was expressed at levels representing greater than 20% of the total *E. coli* protein. However, following cell lysis, all of the fusion protein partitioned into the insoluble fraction and was associated with inclusion bodies. This situation is often the case with heterologous proteins that are expressed at high levels in *E. coli*.

Lowering the culture temperature from 37°C to 25°C during induction of expression resulted in increased levels of soluble fusion protein in the post-sonication supernatant. By increasing the level of soluble protein at this stage, a urea

solubilization and refolding step is avoided. Following clarification by centrifugation, the post sonication soluble supernatant was applied to a Ni^{+2}-NTA agarose affinity column (QIAGEN) and bound protein was eluted with increasing steps of imidazole. The thioredoxin-$MSP1_{42}$ protein from these cells appeared to be properly folded since the recombinant proteins were reactive with mAb 5.2 ($MSP1_{19}$-specific conformation-dependent mouse mAb) on immunoblots. Our data suggested that this expression system could provide sufficient levels of recombinant protein for development as a vaccine antigen.

A second construct was designed [Construct #2, designated pET42(43)] to delete the *E. coli trxA* gene from Construct #1 (thioredoxin-$MSP1_{42}$ fusion, pTRX42). This product was developed as an alternative to the full-length thioredoxin fusion to address potential regulatory concerns with a thioredoxin-$MSP1_{42}$ fusion protein vaccine. Construct #2 simply removes the *E. coli trxA* gene using two *NdeI* sites flanking the gene sequence while maintaining the appropriate reading frame for protein translation of downstream C-terminal $MSP1_{42}$. The product formed retains the His_6-tag for affinity purification and contains some additional vector encoded sequence which includes two proteolytic cleavage sites (approximately 50 amino acids) fused to the N-terminus of $MSP1_{42}$. The N-terminal non-$MSP1_{42}$ 50 amino acids code for pET32a vector DNA sequences, the His_6-tag sequence, an enterokinase cleavage site, an S-peptide tag, and the thrombin cleavage site. The levels of expression from this construct were estimated to be approximately 5-10% of the total *E. coli* protein from crude cell lysates and protein was purified to near homogeneity (>85%) with two consecutive passes over a Ni^{+2}-NTA agarose resin.

Since the levels of expression and apparent protein folding of Construct #2 suggested that a correctly folded non-thioredoxin-fused $MSP1_{42}$ was expressible, a third construct was developed to remove the entire vector-encoded non-MSP1 sequence fused at the N-terminus $MSP1_{42}$ in Construct #2. The entire non-$MSP1_{42}$ sequence upstream of the $MSP1_{42}$ gene sequence was deleted using *NdeI* and *BamH*I and replaced with an annealed oligonucleotide linker to regenerate the His_6-tag and this construct was designated pET42A (Construct #3). Figure 2 shows the general configuration of each linker fused at the N-terminus of the $MSP1_{42}$ gene for Constructs #1, #2, and #3. Therefore, Construct #3 contains a total of 18 non-$MSP1_{42}$ amino acids that encode 6 Histidines and 12 linker amino acids. The non-fused $MSP1_{42}$ molecule from this construct is produced at levels estimated to 2-5% of total *E. coli* proteins from crude cell extracts and the product formed is correctly folded based on immunoreactivity with a series of $MSP1_{19}$-specific mAbs. Potential regulatory concerns over selection in the presence of ampicillin resulted in a further modification of the His_6-$MSP1_{42}$ construct, pET42A (Construct #3) that resulted in the inclusion of the gene for tetracycline resistance, yielding the new construct designated pET42AT#24 (Construct #4). Therefore, the plasmid designated as Construct #4 is identical to Construct #3, except that it also carries the gene for tetracycline selection. The pET42AT#24 construct can be selected in the presence of tetracycline alone during large-scale fermentation or with ampicillin, as necessary. Finally, the pET42AT#24 construct (Construct #4) was further modified to eliminate a Factor X cleavage site present at the N-terminus of the $MSP1_{42}$ gene-cloning site. The Factor X cleavage recognition site was originally introduced into the $MSP1_{42}$ (3D7) sequence by PCR and was not

intended to be a part of this construction. The deletion of this cleavage recognition site was accomplished by removing the entire upstream non-MSP1 sequence using unique *NdeI* and *KpnI* sites. Synthetic oligonucleotides were designed to contain *NdeI* and *KpnI* flanking restriction sites for sub-cloning and coded for a linker sequence of 6 histidine residues followed by a series of glycine and serine amino acids [Construct #5, pET42AT(NK2)] (Figure 3). The expression levels induced from clone pET42AT(NK2) are significantly higher than from Construct #4, pET42AT(#24). This construction should yield higher levels of recombinant protein during downstream fermentation and processing.

All the above described constructs were designed and developed so that affinity chromatography using Ni^{+2} chelating resins would provide simple, highly specific elution profiles of the desired target proteins. The six consecutive histidine amino acid residues were expressed as short N-terminal fusions on the target protein which allowed for specific binding to divalent cations, *i.e.* nickel immobilized onto agarose matrices. The application of cleared soluble supernatant fractions onto the matrix allowed separation of other *E. coli* proteins from the recombinant His_6-$MSP1_{42}$. Only the His_6-$MSP1_{42}$ target protein bound tightly to the resin and was eluted rather specifically using increasing concentrations of imidazole. The specific binding of histidine tagged proteins was through the imidazole ring in the histidine residues to nickel ions immobilized by the NTA (nitrilotriacetic acid) groups on the resin. Increasing concentrations of imidazole compete with the His_6-$MSP1_{42}$ protein for binding to the matrix and result in elution of the tagged protein. The advantage of affinity purification was that target proteins are eluted under gentle, native conditions with relatively few chromatographic steps.

The final purified His_6-$MSP1_{42}$ protein will be analyzed for identity and purity using SDS-PAGE and Coomassie Blue staining for total protein. Proper folding and disulfide bond formation of the recombinant molecules will be measured with a series of $MSP1_{19}$-specific conformation-dependent, reduction-sensitive mAbs and an $MSP1_{33}$-specific mAb on Western Blots or by ELISA's. These mAbs were developed against native parasite lysates and therefore they are appropriate measures of native MSP1-conformation for specific epitopes on C-terminal MSP1.

The above described recombinant $MSP1_{42}$ products were analyzed for correct structure using a series of conformation-dependent mAbs specific for epitopes on either $MSP1_{19}$ or $MSP1_{33}$. Additionally, some recent unpublished data suggests that MSP1 binds to glycophorin-A, an erythrocyte-associated antigen and to a second parasitic surface antigen, EBA-175 (erythrocyte binding protein) forming a three protein complex through some yet undetermined interactions (personal communication and unpublished observation; Dr. Christian Ockenhouse, WRAIR). Thus indirect measures of His_6-$MSP1_{42}$ structure include measurement of their ability to form correct contacts between these protein complexes, either His_6-$MSP1_{42}$/glycophorin-A, His_6-$MSP1_{42}$/EBA or His_6-$MSP1_{42}$/glycophorin-A/EBA (GEM). Preliminary data shows that crude samples of the expressed product from clone pET42AT(NK2) bind to glycophorin and to EBA. The *E. coli* expressed recombinant $MSP1_{42}$ products avoided some of the difficulties in protein folding seen previously for a yeast-expressed recombinant $MSP1_{19}$. The presence of the native N-terminal sequences from $MSP1_{42}$ ($MSP1_{33}$) may have facilitated proper folding and disulfide bond formation by initiating early proper folding pathways during protein translation. In addition, this *E. coli* expression system appears to have

circumvented some problems encountered previously with other bacterial expression systems, mainly proper disulfide bond formation, and partitioning of recombinant proteins into insoluble inclusion bodies.

Optimization of His_6-$MSP1_{42}$ expression requires extensive investigation of variables that affect the efficiency of fermentation and induction of expression. The culture conditions and induction of expression have significant effects on target protein yields and bear upon decisions for downstream purification strategies. Optimal fermentation processes applied to large-scale fermentation can be empirically derived from small-scale culture conditions. Composition of the culture media can have significant effects on the levels of protein produced and the total cell mass.

Specific antibodies to recombinant His_6-$MSP1_{42}$ will be developed by immunization of small animals *i.e.* rabbits, mice. Several *in vitro* assays are available to assess the specificity's of the antibodies developed to recombinant $MSP1_{42}$. First, an indirect fluorescent antibody assay (IFA) measures recombinant antibody binding to native parasite-specific epitopes. In this assay, fixed parasites from native parasite lysates are reacted with antibodies raised against the recombinant His_6-$MSP1_{42}$ and fluorescein isothiocyanate-conjugated secondary antibodies [McBride, *et al.*, 1985]. Positive reactivity with immobilized parasites would suggest that the antibodies induced to His_6-$MSP1_{42}$ recognize some conformationally pertinent epitopes. Alternatively, antibodies raised to recombinant His_6-$MSP1_{42}$ may inhibit the GEM complex formation, again suggesting that the recombinant His_6-$MSP1_{42}$ molecules used to induce these antibodies contain some correct structure or conformation. A parasite invasion-inhibition assay measures the effect of His_6-$MSP1_{42}$ induced antibodies on the *in vitro*

invasion of erythrocytes by merozoites [Holder, *et al.*, 1990]. The ability of antibodies to recognize and bind native MSP1 on parasites and inhibit the re-invasion process would suggest that correct surface epitopes were formed on the recombinant His_6-$MSP1_{42}$ molecules. Antibodies which inhibit erythrocyte invasion, recognize specific epitopes on the C-terminal $MSP1_{19}$ fragment of MSP1, and these antibodies have been shown to prevent the secondary processing event which is necessary for erythrocyte invasion ($MSP1_{42}$ to $MSP1_{33}$ and $MSP1_{19}$). Concomitantly, the ability to inhibit the secondary proteolytic processing event by His_6-$MSP1_{42}$-induced antibodies would provide another useful measure of efficacy. A quantitative assay to test for antibody-induced inhibition of secondary processing events of $MSP1_{42}$ uses merozoites incubated in the presence of radiolabelled antibodies from animals immunized with His_6-$MSP1_{42}$ [Patino, *et al.*, 1997]. The presence of proteolytic products is visualized by autoradiography of probed blots. Therefore, the induction of these invasion-inhibitory antibodies that function via inhibition of $MSP1_{42}$ secondary-processing events should lead to a protective immune response. Finally, inhibition of parasite growth in culture can occur in the presence of specific antibodies that are able to bind to parasite surface determinants and eliminate their ability to associate and invade new red blood cells.

CONCLUSIONS

The data show that the recombinant products formed from the *E. coli* expression system described above, yield recombinant proteins that have some correct conformation. The final *E. coli* expressed His_6-$MSP1_{42}$ product, from pET42AT(NK2) (Construct #5) will be produced at the 10-liter scale to investigate the requirements for

optimal expression and scale-up requirements. Optimized protocols for expression and purification are currently being developed and will be submitted to Forest Glen, Division of Biologics Research, Forest Glen Annex, WRAIR, for future scheduled large-scale fermentation (30 liters) and protein purification. Once recombinant $MSP1_{42}$ has been produced to adequate cGMP levels, it will be assessed for *in vivo* efficacy. Again, the final His_6-$MSP1_{42}$ product will be analyzed against a series of $MSP1_{19}$-specific mAbs to investigate the nature of the protein's conformation. Immunizations with this product may ultimately induce appropriate antibody responses and yield protective immunity. Immunization of non-human primates and human volunteers in Phase I clinical trials will be used to evaluate levels of protection following challenges with the homologous parasites, *P. falciparum* 3D7. However, further characterization of the final $MSP1_{42}$ product derived from Construct #5, pET42AT(NK2), are ongoing and required to assess its feasibility for use as a malaria vaccine antigen.

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Structural Analysis of Refolded-Recombinant *Plasmodium falciparum* MSP1 C-Terminal Fragment By Using Conformation-Specific Monoclonal Antibodies. Evelina Angov[1], Jana S. McBride[2], David C. Kaslow[3], W.R. Ballou[1], Carter L. Diggs[4], and Jeffrey A. Lyon[1]. [1]Dept. Immunology, WRAIR, Washington, D.C., 20307; [2]Division of Biological Sciences, Univ. Edinburgh, EH9 3JT, U.K.; [3]NIAID, NIH, Bethesda, MD 20892; [4]USAID, Washington, D.C., 20523.

Figure 1 pET32a(+) Expression Vector

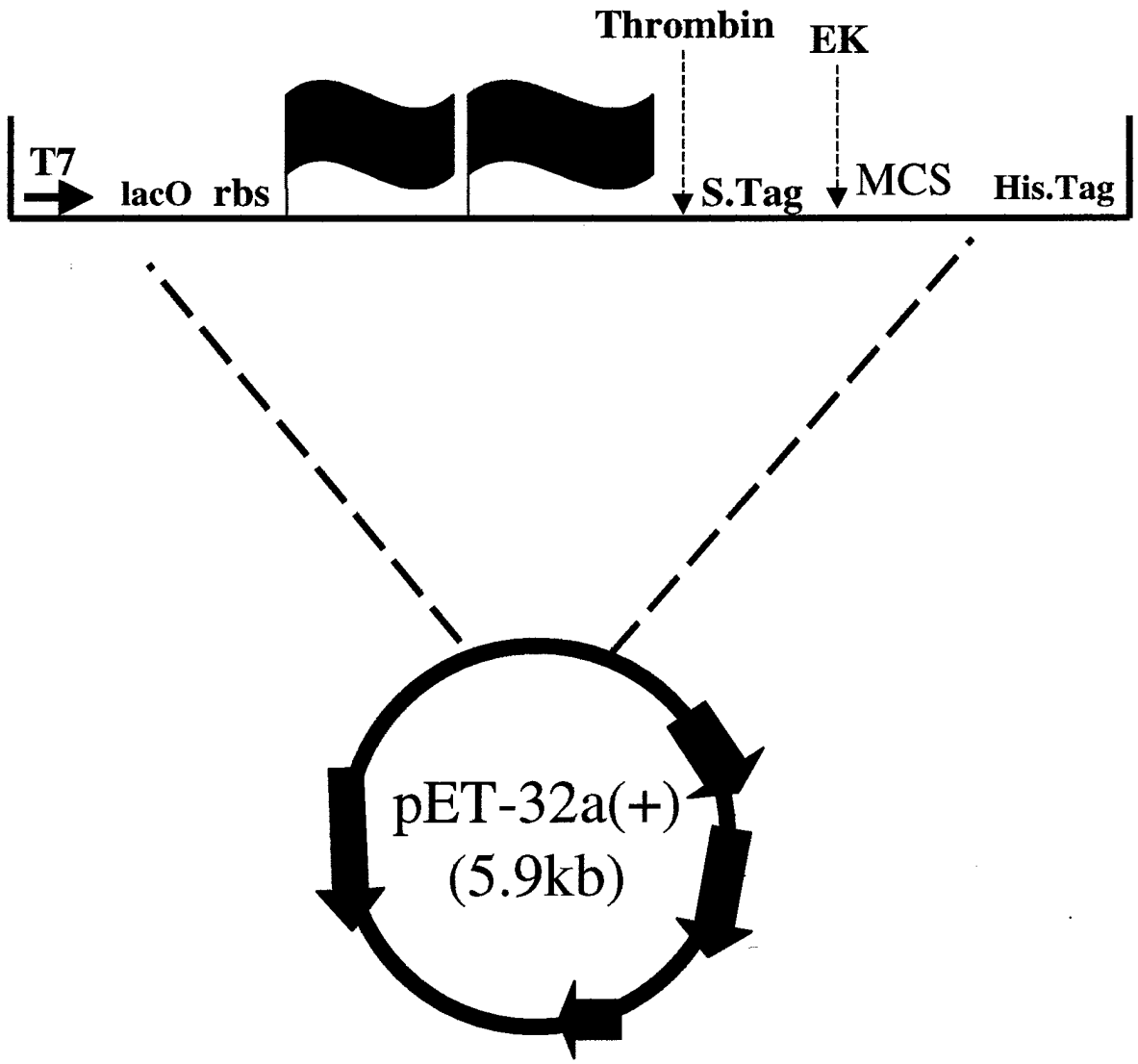


Figure 2 $\underline{MSP1_{42}}$ Constructs

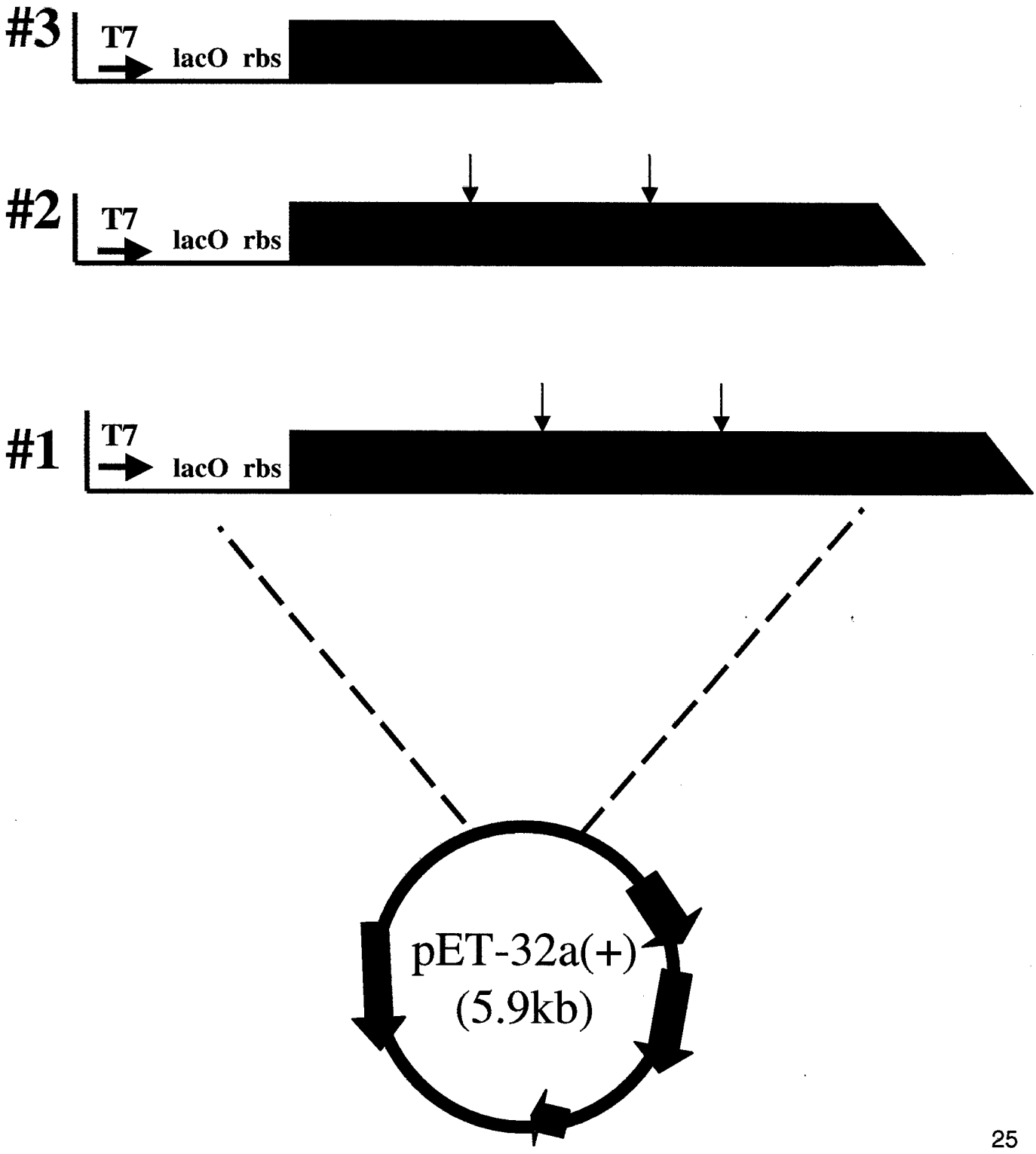
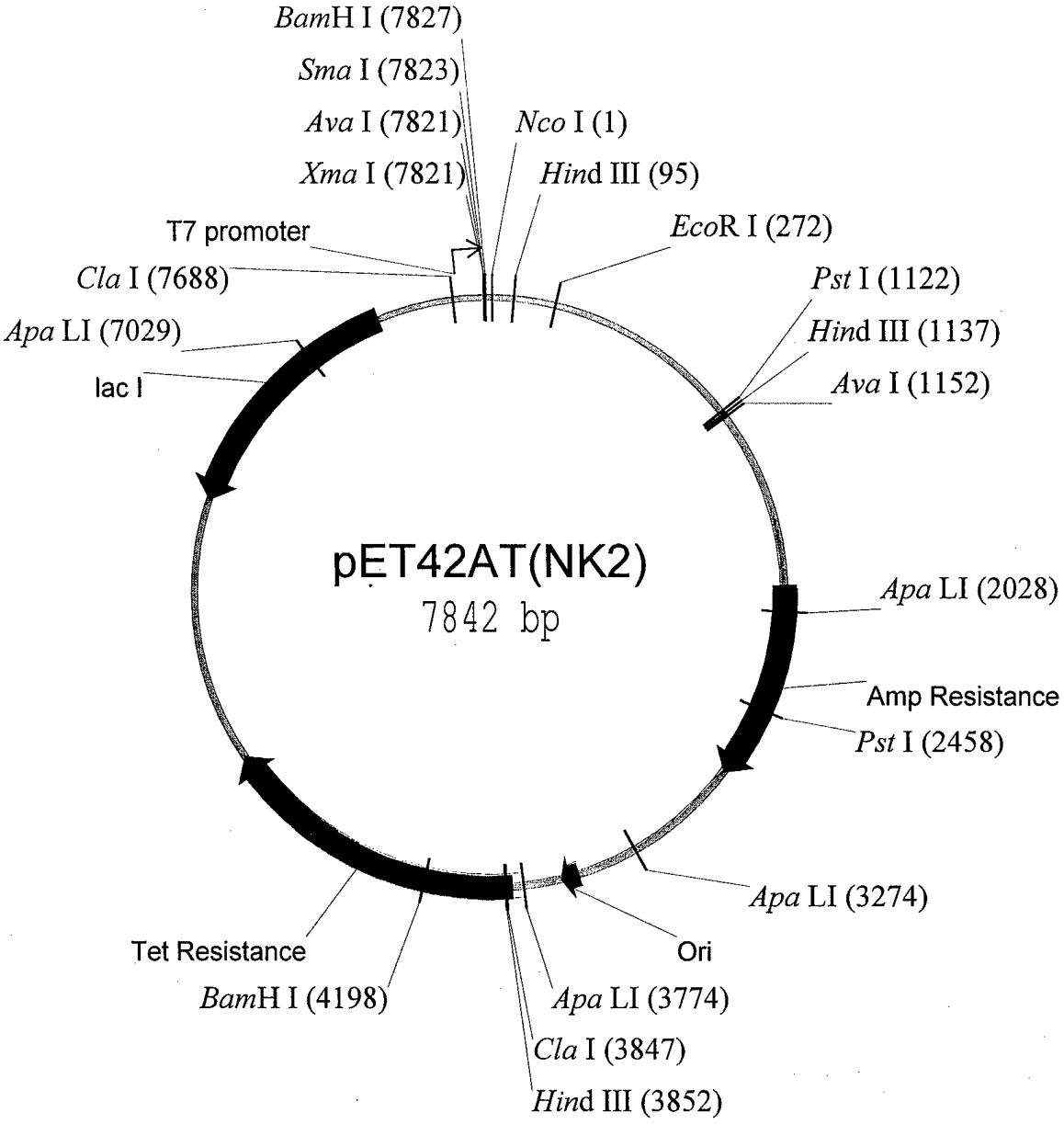


Figure 3


BamH I (7827)
Sma I (7823)
Ava I (7821)
Xma I (7821)
Nco I (1)
Hind III (95)
EcoR I (272)
Pst I (1122)
Hind III (1137)
Ava I (1152)
Apa LI (2028)
Amp Resistance
Pst I (2458)
Apa LI (3274)
Ori
Apa LI (3774)
Cla I (3847)
Hind III (3852)
BamH I (4198)
Tet Resistance
Cla I (7688)
Apa LI (7029)
lac I
T7 promoter
pET42AT(NK2)
7842 bp